NO. 624

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
New York, N. Y. 10017

Application for Research Grant

Date: July 11, 1967

1. Name of Investigator (s):

   John E. Noakes, Senior Scientist, Ph.D.

2. Institution & Address:

   Oak Ridge Associated Universities
   Post Office Box 117
   Oak Ridge, Tennessee 37830

3. Short Title of Project: "Po-210 in Tobacco"

4. Proposed Starting Date: September 1, 1967

5. Anticipated Duration of this Specific Study: Phase I (1 year), Phase II (2-3 years)

6. Brief Description of Objectives or Specific Aims:

   See Attachment

7. Give a Brief Statement of your Working Hypothesis:

   See Attachment

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

   See Attachment

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

   See Explanation on Page 2

10. Additional Requirements:

    A multichannel analyzer is needed for pulse height analysis in the radiometric determination of Ra-226, Pb-210 and Po-210. This instrumentation is available at the Special Training Division for short-term research projects, but teaching activities take priority. It is felt that the extensive use that would be made of this instrument in the proposed tobacco research would necessitate full-time uninterrupted use. In addition to the above equipment, one pH meter and two magnetic hot plates would be required. The present greenhouse would have to be enlarged and heat and lights installed in the greenhouse.

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11. Biographical sketches of all principal and professional personnel (append)

See Attachment

12. List of publications: (Five most recent as pertinent) (append)

See Attachment

9. Physical Facilities Available (continued from page 1)

Oak Ridge Associated Universities, until January 1, 1966, known as Oak Ridge Institute of Nuclear Studies, is a non-profit organization operated by 41 Southeastern U. S. universities serving as a prime contractor in educational, medical, and research activities for Oak Ridge Operations of the Atomic Energy Commission.

All facilities for the performance of the work proposed are owned by the Government and are operated by Oak Ridge Associated Universities for the Atomic Energy Commission under a cost-type management contract. Some of the necessary staff are also employed primarily for such AEC work. The AEC facilities may be used only for the performance of AEC programs, except as the AEC may approve the accomplishment of other work. The terms of our AEC contract contemplate the possibility of our performing such work when agreement that we do so has been reached with the AEC.

Should this proposal receive favorable action and the requested grant be made, agreement will be reached with the AEC regarding the manner in which the grant funds will be applied twoard the cost of the work.

R: REDACTED MATERIAL

13. Budget: (1st year)

	% Time	Amount
A. Personal Services:		
Professional		
Dr. John Noakes	30%	R
Technical		
Technician (Senior Grade)	70%	R
Sub-Total		R
B. Consumable Supplies		
Chemicals		
Radioactive Standards		200
Glassware		100
Silverfoil		200
Platinum discs		100
		150
Sub-Total		$ 750
C. Other Expenses		
Travel and Consultation		$ 750
Secretarial Help		300
Space and Facilities Use		3,100
Sub-Total		$ 4,150
D. Permanent Equipment		
128 Multichannel Analyzer		$ 6,500
Greenhouse Addition Including Heating & Lighting Equipment		2,500
pH Meter & Electrodes (1)		300
Magnetic Hotplates (2)		200
Sub-Total		$ 9,500
E. Overhead on A + B + C,		$ 4,400
Total		$30,900

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Perman. Exp.	Overhead	Total
Year 2	R	2,000	7,500	19,000	8,700	$61,200
Year 3		2,000	7,500	19,000	8,700	$61,200

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants are Made."

Signature John Noakes
Project Director

Signature William G. Pollard
Executive Director

Telephone 483-8411, Ext. 202

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
* Environmental Radiation Studies	AEC	$30,000/yr.	Thru 6/30/68
** Mechanisms of Radiation Injury Studies related to treatment of radiation injury Metals metabolism and medical radioisotope development, Therapy with radiation, Radioisotopes in diagnosis, Biologically important radioisotopic materials: Absorption, Whole Body Retention and Deposition in Specific Organs; NASA Study on radiation effects, Instrument development and methodology			

* Research by Special Training Division

**Research by Medical Division - while not directly related to the first phase of the proposed project, later work in this project will involve the Medical Division.

Pending

11a. Biographical sketches of all principal and professional personnel (append)

John E. Noakes

Birthplace: R

Date of Birth: R

Education: B.S. - Champlain College - 1953
Graduate Study - Syracuse University
M. S. - Texas A & M University - 1959
Ph.D. - Texas A & M University - 1962

Member: Professional
American Chemistry Society
Honorary
Sigma XI

Academic Experience:

1962-Present: Senior Scientist, Special Training Division, Oak Ridge Associated Universities, senior lecturer with research in geochronology of marine sediments and environmental studies.

1961-1962 Assistant Professor (Research), Institute of Marine Science, University of Alaska; work concerned with establishment of a marine laboratory and research in clay minerology.

1960-1961 Research Scientist II, Texas A & M University; responsible for setting up and operating the carbon-14 dating laboratory using the liquid scintillation method.

1958-1960 Research Chemist, Texas A & M University; IGY Program, "Mohole" Investigation, and course instructor.

1957-1958 Research and teaching assistant and work in development of an analytical method for boron evaluation in marine waters; and investigation of long chain unsaturated fatty acids.

1955-six months Graduate Assistant at Syracuse University

Industrial Experience:

1959-1962 Served as a consultant for toxicity work applied to industrial application.

1955-1957 Research Chemist and Junior Executive position, Clark-Cleveland Company. Work in product development in the field of plastics.

Personal contact with Battelle Memorial and Evans Research Centers with laboratory evaluation of their submitted data.

1953-1955 Soils Engineering - New York State, Soil Analyst

1965-Present Consultant - Picker Instrument Company

Patents: Low Temperature Conversion of Acetylene to Pure Benzene #424804

Publications: "The Distribution of Boron and Boric Acid Complexes in the Sea," Master Thesis, Texas A & M University, 1959

"Boron-Boric Acid Complexes in Sea Water," presented at the International Union of Geodesy and Geophysics, Helsinski, Finland, 1960 and Noakes, J. E. and D W. Hood, Jour. of Deep Sea Research, 8, 121-129, 1961.

"C^{14}/C^{12} Ratios of the Organic and Inorganic Carbon Fraction of Waters of the Caribbean and Gulf of Mexico," Final Report A & M, Project 235, N.S.F. Grant-610232, 15 February 1961.

"Carbon Dating by Liquid Scintillation," Noakes, J. E. and D. W. Hood, presented at the Oak Ridge Special Course, "Nuclear Methods as Applied to Oceanography," November, 1961.

"Benzene Synthesis Acids C^{14} Dating," Chemical and Engineering News, October 9, 1961.

"Conversion of Carbon Dioxide to Benzene for Liquid Scintillation Counting," Noakes, J. E., A. S. Isbell, and D. W. Hood, Trans. Amer. Geophys. Union, 42, No. 2, 226, 1961.

"Univ. of Texas Radiocarbon Dates I," Stipp, J. J., E. M. Davis, J. E. Noakes, and T. E. Hoover, Amer. Jour. of Sci. Radiocarbon, Suppl. 4, 1962.

"Natural Radiocarbon Measurements by Liquid Scintillation Counting," Dissertation, Texas A & M University, 1962.

"Low Temp Benzene Synthesis for Carbon-14 Dating," Noakes, J. E., A. F. Isbell, J. J. Stipp and D. W. Hood, Geochemica et Cosmochimica Acta, 27, No. 7, 797-804, 1963.

"Texas A & M University Radiocarbon Dates I," Noakes, J. E., J. J. Stipp, and D. W. Hood, Amer. Jour. of Sci. Radiocarbon Suppl., 6, 189-193, 1964.

"Geochronology of the Gulf of Mexico, Part I," Rona, E., L. K. Akers, J. E. Noakes, I. R. Supernaw, Progress in Oceanography, Vol. 3, Perganon Press, 1965.

Publications (Cont'd)

"A Chemical Study of an Ambient Temperature Catalytic Benzene Synthesis Used in Radiocarbon Dating, Noakes, J. E., S. M. Kim, G. A. Thomas, and L. K. Akers, ORINS Publication #46, November, 1964.

"Cobalt Molybdate Catalyst for Ambient Temperature Synthesis of Benzene for Liquid Scintillation Radiocarbon Dating," Noakes, J. E., S. M. Kim and L. K. Akers, ORINS-50, April, 1965.

"Chemical and Counting Advances in Liquid Scintillation Counting," J. E. Noakes, S. M. Kim and J. J. Stipp, Pullman Conference - June 7-11, 1965, Paper presented and published in proceedings. U.S.A.E.C. Conf.-650652

"Electrodeposition of Actinides and Lanthanides," Kim, S. M., John E. Noakes, L. K. Akers, W. W. Miller, ORINS-48, December 15, 1965.

"Electrodeposition Method for Counting Alpha and Beta Emitters," Kim, S. M., J. E. Noakes, and W. W. Miller, Nucleonics, 24, #3, March, 1966.

"Anomalies in the Th^{230}/Th^{232} Activity Ratio in Some Mississippi River Sediments, Noakes, J. E. and I. R. Supernaw, (presented at AGU Meeting in April, 1965. Journal Geophysical Research (in press)

"Recent Improvements in Benzene Chemistry for Radiocarbon Dating," J.E. Noakes, S. M. Kim and L. K. Akers, Geochimica and Cosmochimica Acta (in press)

"Oak Ridge Institute of Nuclear Studies Radiocarbon Dates I," Noakes, J. E., S. M. Kim and L. K. Akers, Jour. of Sci. Radiocarbon Suppl. (in press)

"The Mass of a Neutron: A Student Exercise", H. E. Banta and J. E. Noakes, American Journal of Physics (accepted for publication)

"Uranium Content of Gulf of Mexico Sea Water", J. E. Noakes, S. M. Kim and L. K. Akers, Presented at American Geophysical Union, April, 1967 (to be published)

"A New Method for Thorium Analysis from Marine Sediment," S. M. Kim, J. E. Noakes, Presented at American Geophysical Union, April, 1967 (to be published)

(6) Objectives of Proposed Po-210 Tobacco Research

The first phase of the program would be a geochemical study of the sources of Po-210 which might be available to tobacco plants and the gross mechanism by which Po-210 could enter the plant system. The second phase would be a medical study to determine if and at what concentration levels Po-210 in biological systems could be considered to be carcinogenic.

Specific Aims on a Yearly Basis

(a) Identification of parent radioactive source material most responsible for the occurrence of Po-210 in tobacco (Ra-226, Rn-222, Pb-210).

(b) Mechanism by which Po-210 parent finds its way into the plant system (air-plant interface, soil-root interface, etc.).

(c) Establish conditions for decreasing Po-210 content in tobacco plants grown in a natural environment (phosphate fertilizer, soil pH control, etc.).

(d) Verify controlled conditions for growing tobacco with low and high concentrations of Po-210 for medical studies.

Second Year

(a) Bulk amounts of tobacco grown with high and ultra low Po-210 content under conditions established from first year study.

(b) Bulk tobacco of high and low Po-210 content analyzed for trace metal content, tars, nicotine, etc., to be used as standardized reference tobacco sources for biological studies.

(c) Consultation and preliminary evaluation of most suitable biological specimens to be used in medical studies.

(d) Perfection of methods and equipment for introducing tobacco smoke into biological specimens and analytical methods to be employed in the evaluation of results. (c and d - Current tobacco research projects to be evaluated.)

Third Year

(a) Medical studies of biological specimens subjected to high and low concentration of Po-210 tobacco smoke.

(b) Pathological and chemical analysis to be carried out on sacrificed animals to determine Po-210 content in various organs any evidence of alteration in cell development.

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Fourth Year

(a) Continuation of medical investigation looking into specific aspects of third year study which indicates further investigation is warranted.

(7) Working Hypothesis

Radford and Hunt (Science 1964) were first to publish the finding of high concentration of Po-210 found in the respiratory and pulmonary systems of tobacco-smoking individuals. The radiation dose rate in bronchial epithelium for long duration smokers (2 packs a day for 25 years) was calculated to be as high as 200 REM. This value far exceeds the maximum dose of 1.1 REM to an entire lung as recommended by the International Council for Radiation Protection (ICRP). They concluded that the dose was sufficient to cause alteration in cell development and could be an important source for initiating bronchial cancer in smokers. More recent studies have reported Po-210 found in the liver, kidneys, blood, etc., of tobacco-smoking people which has led to supposition that it should be a prime suspect as a cancer forming agent.

Skrable (Science 1964) and others have taken exception to this work and formulated mathematical models to substantiate their statement. Because of the rather limited experimental and analytical data available for the biological half-life of Po-210 and its alpha radiation damage to various organs, no mathematical model can be constructively applied.

The occurrence of Po-210 in tobacco has also raised the question as to the source and mechanism by which this radionuclide finds its way into the plant. Studies by Gregory (Science 1965) on the Po-210 content in tobacco obtained from all parts of the world show a wide variation. U. S. tobaccos rank among the highest in Po-210 content with values of 1-0.5 pc/g of tobacco.

Berger (Science 1965) has attributed the Po-210 content of tobacco as resulting from the assimilation of Rn-222 of gas at the plant-atmosphere interface. Atmospheric Rn-222 concentration is between 50-200 pc/m^3 and originates from the decay of Ra-226 in ground materials. Berger's work has concentrated on showing that the major portion of the Po-210 uptake in tobacco occurs after harvest and during the curing period.

Tso (Science 1966) has attempted to refute this work by showing that a more plausible explanation for Po-210 in tobacco is in plant root uptake of Pb-210 from the soil. He demonstrated in his studies that tobacco plants subjected to high concentrations of Pb-210 for a short growth period can contain Po-210 as high as 150 pc/g of tobacco.

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Po-210 content of tobacco has been of ORAU staff interest since the first reports appeared in the literature. Speculation was that one source of Po-210 in tobacco could be the Florida phosphate rock used as a phosphorous source for essentially all fertilizers in the United States. This phosphate rock is reported to contain 0.01-0.05% uranium and would be expected to have in secular equilibrium, the radioactive daughter Po-210. Radiometric analysis of the major constituents of commercial tobacco fertilizers have revealed that Po-210 occurs in the phosphate rock fraction in concentrations of 20-25 pc/g. The yearly application of high phosphate fertilizer to American tobacco growing soils may well be the prime reason for the high Po-210 found in U. S. tobaccos.

Further studies have been carried out at ORAU to determine if Po-210 free tobacco could be grown in a controlled environment. Tobacco plants grown hydroponically in nutrient solution free of Po-210 or its precursors have been found to contain Po-210 in concentrations of <.01 pc/g of tobacco. These studies and those of Tso, clearly show that Po-210 content in tobacco can be regulated to desired amounts under controlled experimental conditions. These studies also indicate that similar controlled experiments could be devised to determine the source and mechanism for uptake of Po-210 into tobacco plants.

The ability to produce tobacco with regulated amounts of Po-210 also offers potential application to biological studies. Introduction of controlled amounts of Po-210 in smoke to biological specimens would facilitate quantitative evaluation of such parameters as Po-210 uptake, body distribution and retention times. It would also enable comparison of biological specimens possessing low and high Po-210 content for carcinogenic evaluation.

(8) Experimental Design

The experimental procedures proposed for accomplishing the first year's goals set forth in section 6 of this proposal can best be described as ~~three sets of~~ experiments which will be concurrently carried out.

I. Radioactive materials responsible for Po-210 in tobacco plants and gross mechanism of plant uptake.

Three groups of tobacco plants will be greenhouse raised. One group of plants will be grown in quartz sand free of Po-210 or any parent radioactive material (photo 1). The second plant group will be grown in typical virgin Tennessee tobacco soil. The third group of plants will be grown in similar soil, but will have phosphate fertilizer added to them which contains appreciable amounts of Po-210. All plant groups will be nourished with nutrient solution made with reagent grade chemicals free of any Po-210 or parent radioactive material.

All tobacco plants from the three groups will be harvested at maturity. The quartz sand grown tobacco plants should show the lowest Po-210 content of all tobaccos and reflect only the uptake of parent Rn-222 at the atmosphere-plant interphase during time of growing and curing. The tobacco Po-210 content from atmospheric Rn-222 contribution during curing will be evaluated by preferential curing methods.

The Po-210 content of tobacco plants grown in soil with nutrient added will reflect the soil uptake and the Rn-222 contribution. The Po-210 furnished by the soil can be calculated by subraction of the Po-210 contribution from Rn-222.

The Po-210 content of tobacco plants grown in soil with phosphate fertilizer added will be a composite value of Rn-222 uptake, plus soil contribution and fertilizer contribution. Since the Po-210 soil and Rn-222 contribution will be known, the fertilizer Po-210 contribution to the tobacco can be calculated.

It should be pointed out that the Po-210 soil contribution to the tobacco plant at the time of harvest can be composite value of the direct non-supported Po-210 uptake from the soil and parent grow-in. Ra-226, Pb-210 and Po-210 analysis of the mature tobacco at harvest and at later times will permit calculation of the decay of the non-supported Po-210 and the grow in of the Po-210 parent contribution. These analyses will also enable identification of the Po-210 parent which is entering the plant-root system and soley responsible for the Po-210 content in aged smoking tobaccos.

II. Conditions for decreasing Po-210 content in soil grown tobacco plants.

Three groups of tobacco plants will be grown in Tennessee soil under greenhouse conditions. Each plant will receive a known amount of radioactive Pb-210 in equilibrium with its daughter Po-210 and nutrient solution of controlled pH.

The first group of plants will be raised with nutrient solution of pH5. The second group will be raised with nutrient solution of pH7. The third group will have nutrient solution of pH7 with added sulfate ions.

Plants will be grown to maturity and analyzed at harvest time for Pb-210 and Po-210 content. The first group of plants will represent uptake under acid soil conditions which should reflect less tendency of the soil to hold the available Pb-210 and Po-210. The second plant group will represent uptake of Pb-210 and Po-210 from the added radioactive spike and the normal soil contribution. Plants grown in the third group under pH7 soil conditions with excess available sulfate ions should indicate the degree of formation of insoluble Pb-210 sulfate as compared to available Pb-210 for plant uptake.

The presence of high concentrations of sulphate ions in commercial fertilizers is due to the sulfuric acid treatment of phosphate rock during fertilizer manufacturing. The possibility of the formation of insoluble $PbSO_4$ in soil at raised pH should therefore be considered.

Soil pH is known to be a prime factor in affecting plant trace metal uptake. If the tobacco uptake of Pb-210 and Po-210 is found to be altered by soil pH, it may be one explanation for the wide variations in Po-210 content found in tobaccos grown in all parts of the world. It may also reveal advantages to be gained by incorporation of additives to fertilizer or soils to better control soil pH.

III. Pb-210 and Po-210 tobacco uptake studies to establish conditions for growing tobaccos of high Po-210 content.

Three groups of tobacco plants will be grown in quartz sand under greenhouse conditions. All plants will be fed nutrient solution for their entire growth period. Each group of plants, early in its development will be subjected to a designated amount of radioactive Pb-210 in equilibrium with its daughter Po-210. Radioactivity administered to the first group will be 1x, the second group 10x and the third group 100x, where x = μ curie levels.

All tobacco plants will be raised to maturity and analyzed at time of harvest for Pb-210 and Po-210 content. These results will establish the upper levels of Po-210 content which can be obtained in tobacco. Low level Po-210 tobacco data will be supplied from series I experiments. A plot of the Po-210 tobacco uptake with regard to available Pb-210 and Po-210, will be used to establish predictable conditions for growing tobacco of desired Po-210 content to be used in the medical studies.

ANALYTICAL METHODS

I. Tobacco trace metals, tar and nicotine content

Tobacco grown under experimental conditions (photo 2) will be analyzed for trace metals, tar and nicotine content. Similar analyses of commercial tobaccos will be conducted for comparative pruposes. Trace metal analysis will be carried out using non-destructive slow and fast neutron activation analysis. The tar and nicotine analyses will be conducted by various methods.

Bates

II. Radioactive Analyses of Ra-226, Pb-210 and Po-210

Radiometric analysis of Ra-226, Pb-210 and Po-210 are standard methods which are routinely taught and practiced at the Special Training Division of ORAU. The tobacco and fertilizer analyses for these three isotopes are carried out by digestion and dissolution with strong oxidizing agents. Ra-226, and Pb-210 are separated using ion exchange chromatography and electroplated onto platinum discs by applied potential. Po-210 is spontaneously electroplated onto silver discs by its own redox potential. The quantitative yields for the chemical separation and electrodeposition of each of these three isotopes has been determined by radioactive tracers.

Alpha counting is carried out with solid state detectors or ion chambers (photo 3) of measured counting efficiency. Alpha spectroscopy (photo 4) using pulse height analysis is employed for quantitative measurement of these isotopes.

AEC FACILITIES AND EQUIPMENT REQUIRED TO CARRY OUT PROPOSED RESEARCH PROJECT,

"Po-210 in Tobacco"

Facilities & Equipment	Equipment Acquisition Cost	Equipment Acquisition Date	% Time Usage
1. Laboratory, 10' x 10', equipped with the following:			100%
a. Sink with hot and cold running water			
b. Distilled water facility			
c. Bench top, 3'x10'			
d. Fume hood with electrical outlets and water			
e. Storage cabinets and shelves			
f. Desk			
2. Greenhouse, approximately 108 square feet			100% for approximately four months
3. Liquid Scintillation Counter, prop. #8798	$13,825	August, 1965	20%
4. Ion Chamber prop. #7149	$ 669	June, 1959	30%

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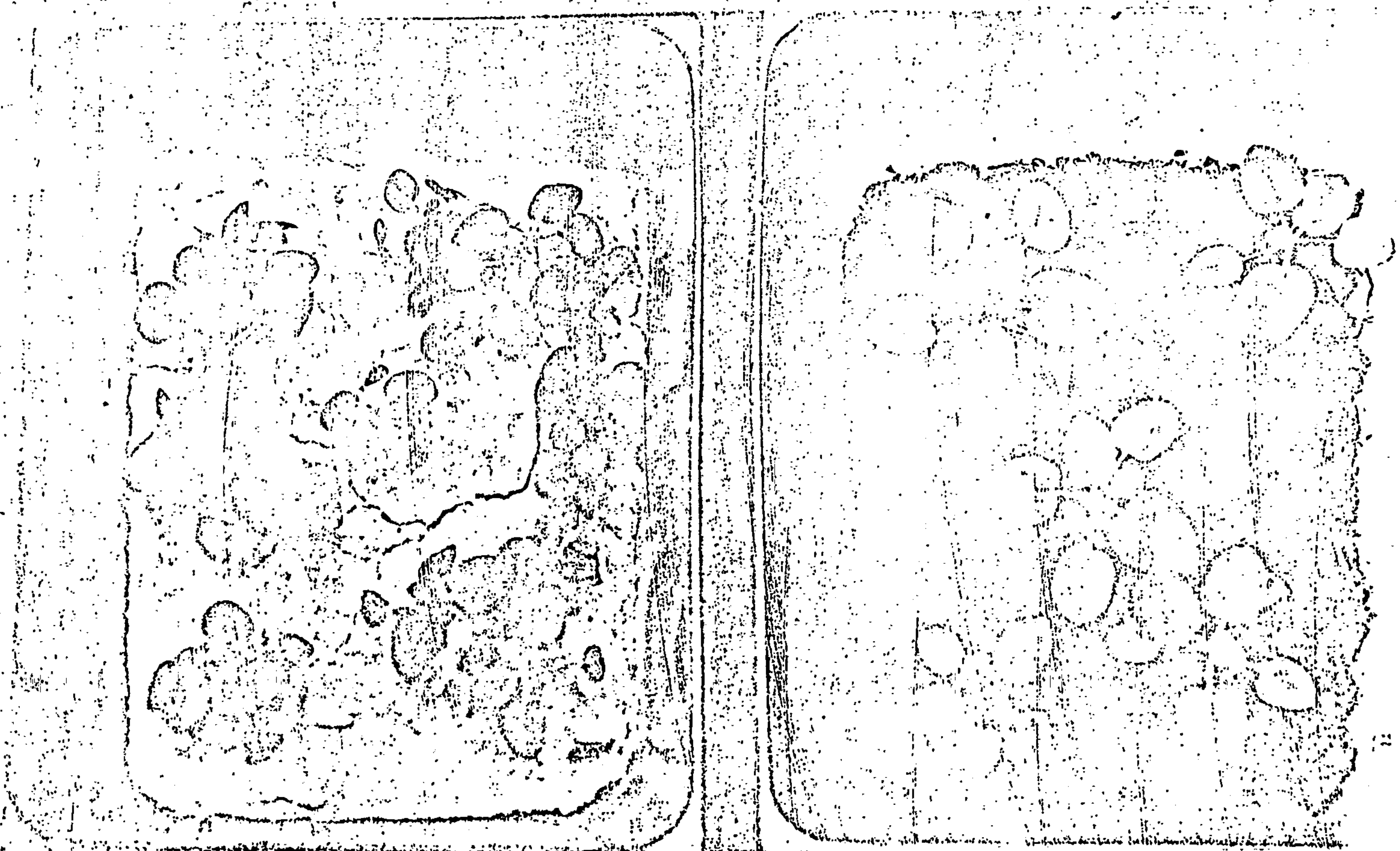
Photo 1
Greenhouse Grown Tobacco Plants

Quartz Sand

Tennessee Soil



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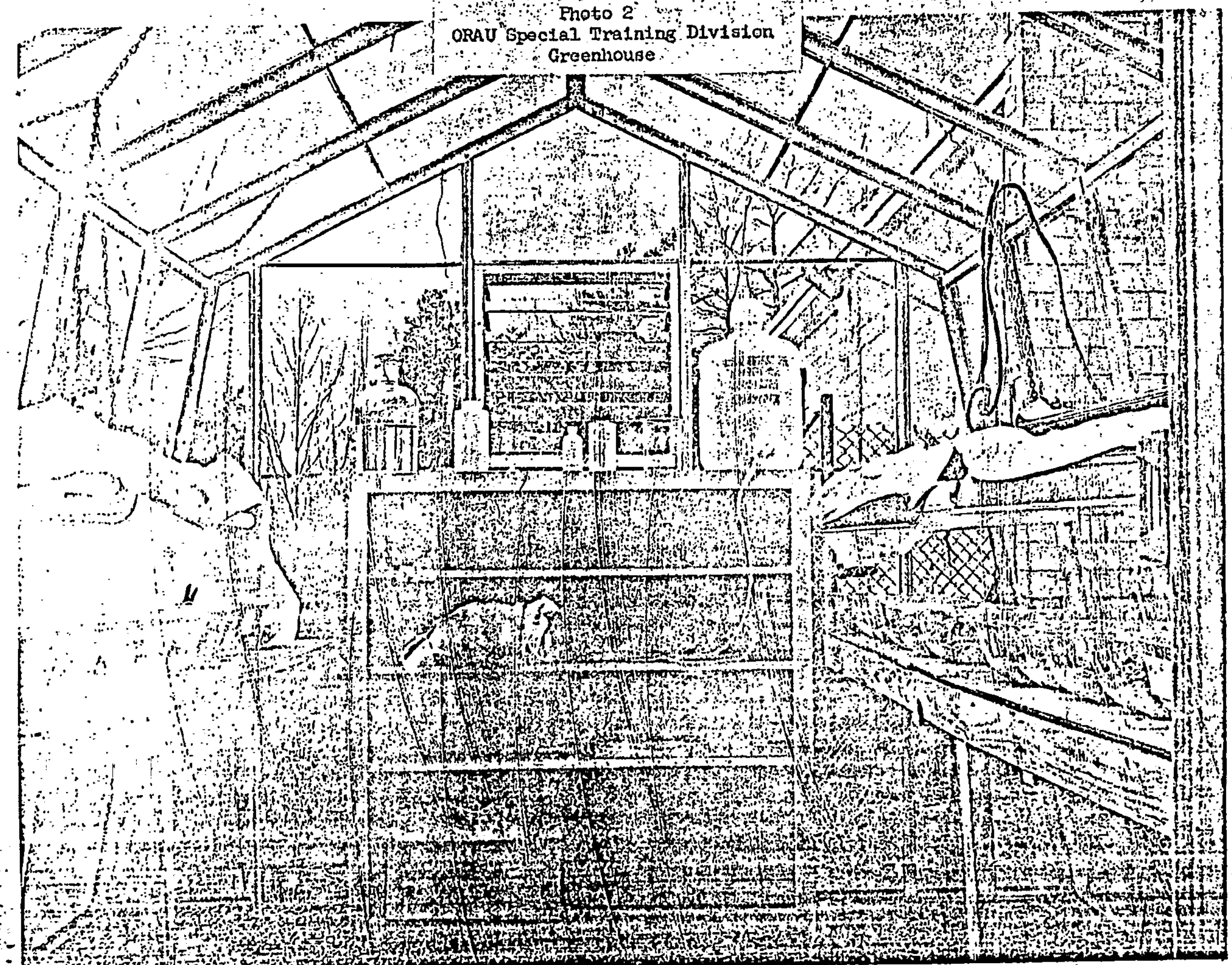

Photo 2
ORAU Special Training Division
Greenhouse

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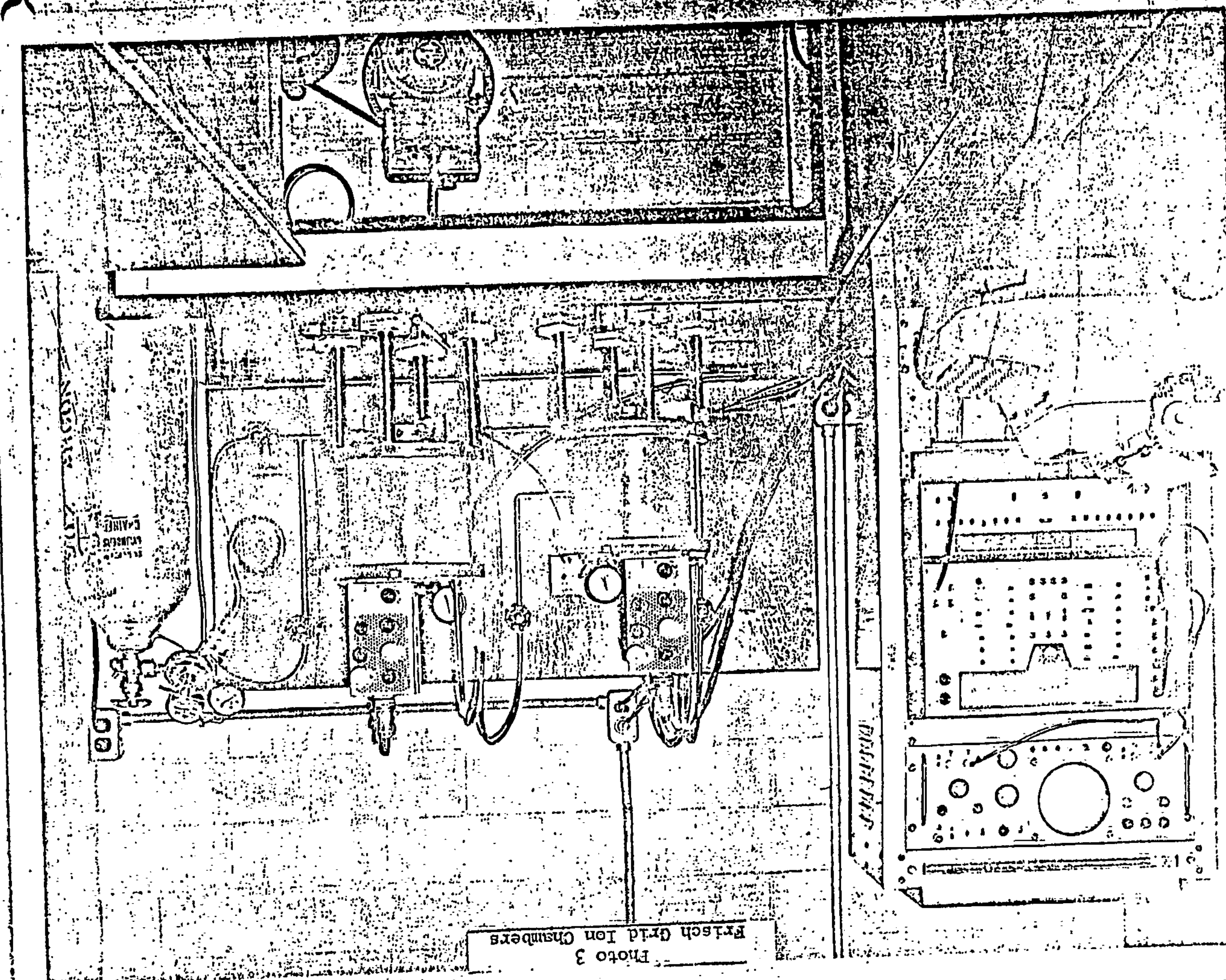
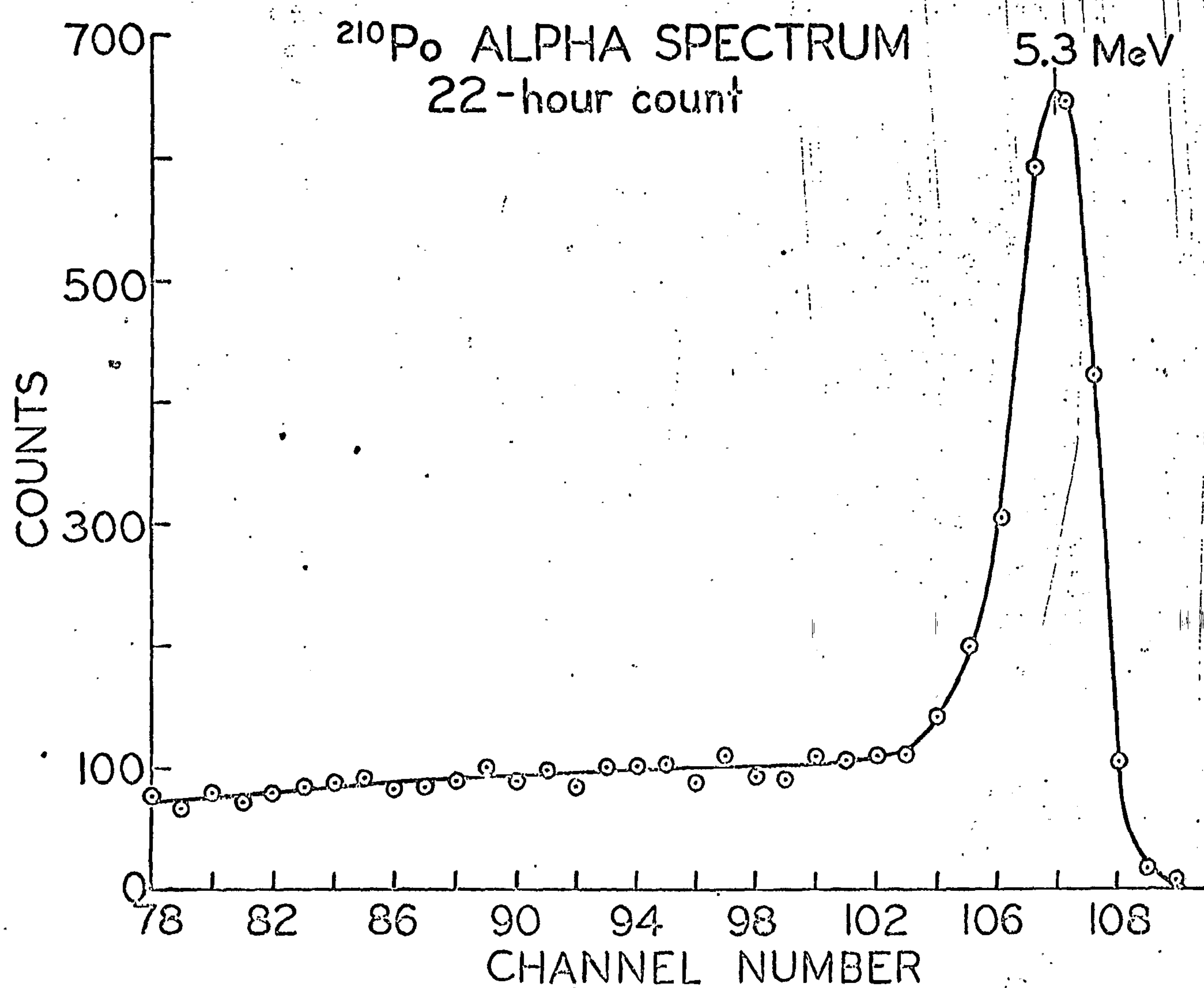


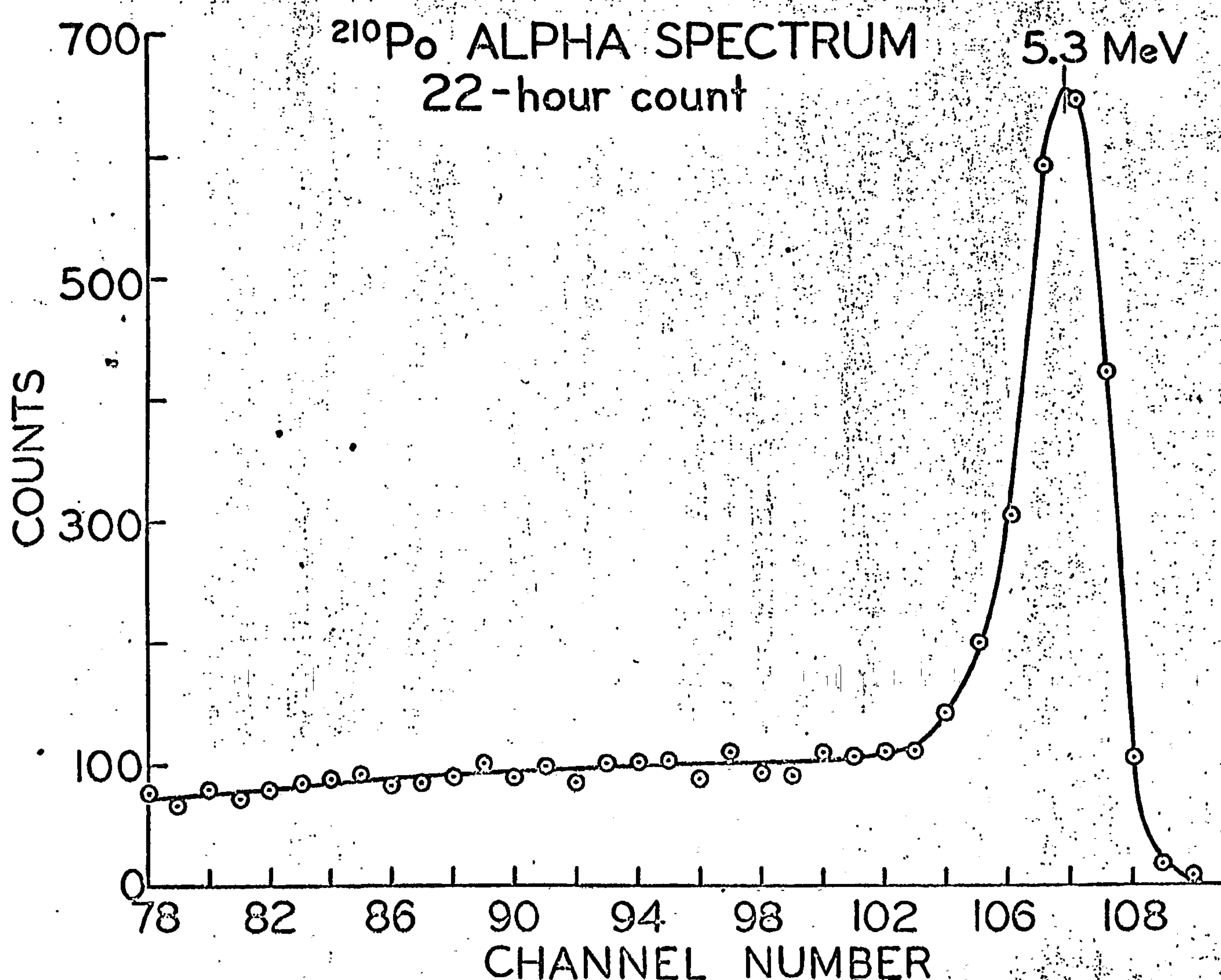
Photo 3
Frisch Grid Ion Chambers

Photo 4



^{210}Po ALPHA SPECTRUM
22-hour count
5.3 MeV
COUNTS
700
500
300
100
0
78
82
86
90
94
98
102
106
108
CHANNEL NUMBER

Photo 4



^{210}Po ALPHA SPECTRUM
22-hour count
5.3 MeV
COUNTS
700
500
300
100
0
78
82
86
90
94
98
102
106
108
CHANNEL NUMBER